Genetic Variation in the Eastern Cottonmouth, *Agkistrodon piscivorus piscivorus* (Lacépède) (Reptilia: Crotalidae) at the Northern Edge of its Range

DONALD A. MERKLE
Department of Natural Sciences, Longwood College, Farmville, Virginia 23901

ABSTRACT.— Genetic variation was examined by electrophoresis in six populations of *Agkistrodon p. piscivorus* from the northern edge of the species' range in southeastern Virginia. Twenty-three presumptive loci were found to be monomorphic, while three loci were polymorphic in some populations. Average observed heterozygosity values ranged from 0.9% to 2.9%, with a mean for all populations of 1.6%. Nei's index of genetic identity reveals that all Virginia populations sampled have a very high degree of genetic similarity, with a minimal value of .964.

INTRODUCTION

Advances in biochemical techniques have enabled researchers to collect a great deal of information on the amount of genetic variation that occurs in natural populations (Selander and Johnson 1973). Most of the studies dealing with snakes have involved the family Colubridae (Sattler and Guttman 1976; Gartside et al. 1977). Those studies that included species of the family Crotalidae analyzed only components of the venom.

The Eastern Cottonmouth, *Agkistrodon piscivorus piscivorus* (Lacépède), occurs primarily in the Coastal Plain of the eastern United States, where it ranges from Alabama to its northern distributional limits in the southeastern corner of Virginia. For years, the James River was thought to mark the northernmost limit for this species, just as it does for a number of other reptiles and amphibians (Wood 1954; Conant 1975). Cottonmouths, however, occur north of the James River in the vicinity of the Newport News-Hampton area (Engeling 1969; Linzey and Clifford 1981).

The distribution of the cottonmouth in Virginia is discontinuous. There is an isolated population along Swift Creek and the Appomattox River just west of Hopewell in Chesterfield County. "This population is at least 60 km from the nearest known locality in the main part of the range. . ." (Blem 1981:117). Populations of this species on the barrier beaches along Back Bay are separated from the mainland by several miles of water. Other populations, such as that at Sea Shore State Park,

are essentially isolated by "urban sprawl". Thus, there appear to be a number of potential barriers to gene flow in populations of this species in Virginia.

Several other species of snakes whose northern limits are essentially restricted to the Coastal Plain of southeastern Virginia also occur on the peninsula between the James and York rivers. They include the Timber Rattlesnake, *Crotalus horridus*; the Brown Water Snake, *Nerodia taxispilota*; the Redbelly Water Snake, *Nerodia erythrogaster erythrogaster*; and the Glossy Crayfish Snake, *Regina rigida rigida* (Linzey and Clifford 1981).

The distribution of such populations is open to two interpretations. The first is to assume that populations occurring north of the James River were established by animals that crossed the river in the same area despite its width (approximately 6 km). The second hypothesis is to assume that all of these species once occurred throughout the Coastal Plain of southeastern Virginia. This would enable colonizers to invade areas north of the James by crossing in the general vicinity of the Fall Line, where the river is a less formidable barrier. Subsequent elimination of intervening populations would then produce the distributional patterns currently observed. The potential isolation of populations could result in genetic differentiation due to lack of gene flow.

In this study, I used electrophoresis to examine genetic variation in several populations of *A. p. piscivorus* to determine: 1) levels of genetic variation that might accompany potential isolation; and 2) if patterns of genetic variation reveal any insights into the origin of populations of *A. piscivorus* north of the James River.

MATERIALS AND METHODS

Specimens collected in the field from May 1980 until September 1982 were returned to the laboratory and sacrificed by freezing. Attempts were made to collect specimens from every drainage system in Virginia where the species has been recorded. Localities where snakes used for this study were collected (Fig. 1) are: 1) Newport News Reservoir, Newport News; 2) Appomattox River near Hopewell; 3) Sea Shore State Park, Virginia Beach; 4) Gum Swamp near North Landing River, Virginia Beach; 5) Northwest River, Chesapeake; and 6) False Cape State Park, Virginia Beach.

Prior to electrophoresis, specimens were thawed and extracts of soluble proteins prepared by homogenizing samples of heart, liver, and skeletal muscle with equal volumes of 2% 2-phenoxyethanol. Homogenates were centrifuged at 4,000 G for 30 minutes. Supernatants were then decanted and used for electrophoresis.

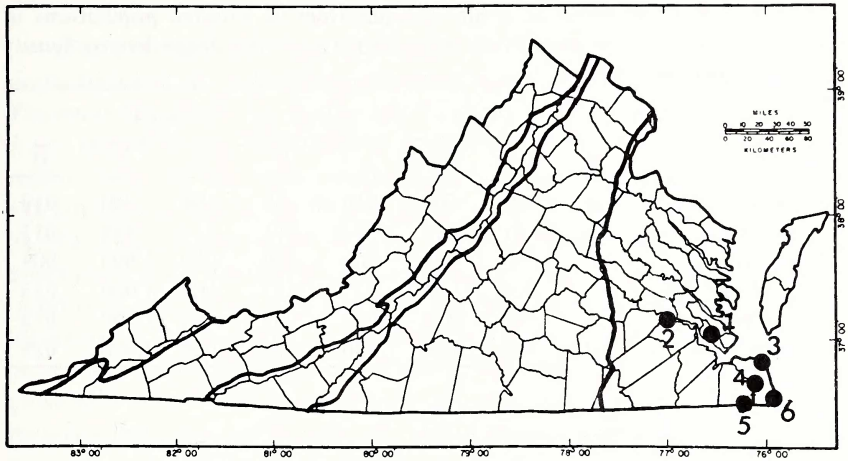

Fig. 1. Populations of *A. piscivorus* sampled for electrophoretic study. 1 = Newport News, 2 = Hopewell, 3 = Sea Shore State Park, 4 = Gum Swamp, 5 = Northwest River, 6 = False Cape State Park.

Starch gel electrophoretic techniques using the basic procedures of Selander et al. (1971) were used with the following modifications: albumin, hemoglobin, general proteins, and esterases were best demonstrated on the Poulik buffer, while all other enzymes were isolated on gels using the Tris-Maleic acid buffer. All gels were 12.5% starch (Electrostarch Lot 307, Otto Hiller, Madison, Wisconsin).

Isozymes of various proteins were designated in order of decreasing anodal mobility. Alleles present at polymorphic loci were designated alphabetically by a superscript following the locus designation. Nei's (1972) index of genetic identity was used to compare genetic similarities between populations, and mean heterozygosity per individual ($\bar{H}$) was calculated for each population.

RESULTS

Of the 29 protein loci examined in this study, 23 were found to be monomorphic in all individuals examined. These loci included: albumin, hemoglobin, three general proteins, three esterases, two malate dehydrogenases, two lactate dehydrogenases, two phosphoglucomutases, one phosphoglucose isomerase, α-glycerophosphate dehydrogenase, glutamate dehydrogenase, two superoxide dismutases, isocitrate dehydrogenase, 6-phosphoglucose dehydrogenase, sorbital dehydrogenase, and glutamate oxaloacetate transaminase (Got-1). Only three loci were polymorphic; their gene frequencies are listed in Table 1.

Table 1. Allelic frequencies at polymorphic loci in Virginia populations of *Agkistrodon piscivorus*. N = sample size; $\bar{H}$ = mean heterozygosity per individual.

		Got-2		Xdh-1		Lap-1		
Locality	N	a	b	a	b	a	b	$\bar{H}$
1. Newport News	10	.300	.700	.850	.150	.100	.900	.019
2. Hopewell	9	.111	.899	.944	.056	.111	.889	.017
3. Sea Shore	2	.250	.750	.000	1.000	.000	1.000	.009
4. Gum Swamp	7	.214	.786	.857	.143	.000	1.000	.013
5. Northwest R.	11	.364	.636	.091	.909	.000	1.000	.012
6. False Cape	10	.400	.600	.350	.650	.000	1.000	.029

Variability at Polymorphic Loci

Got-2: This locus was polymorphic in all populations, with the frequency of Got-2^a ranging from .111 in the Hopewell population to .400 in the False Cape State Park population.

Xdh-1: Two alleles were present in all populations, with the exception of Sea Shore State Park where only Xdh-1^b was present. Xdh-1^b was the predominant allele in the False Cape State Park and Northwest River populations, while Xdh-1^a predominated in the populations from Hopewell, Newport News, and Gum Swamp.

Lap-1: Most individuals were monomorphic for Lap-1^b, but one individual from both Newport News and Hopewell populations possessed Lap-1^a in the heterozygous condition.

Observed mean individual heterozygosity values ($\bar{H}$) ranged from a low of 0.9% in the Sea Shore State Park population to a high of 2.9% in the population at False Cape State Park. The mean heterozygosity per locus per individual for all populations was 1.6%. Nei's (1972) index of genetic identity values for all pairings is presented in Table 2.

Table 2. Nei's index of genetic identity values between populations of *Agkistrodon piscivorus* from Virginia.

	Newport News	Sea Shore	Gum Swamp	Northwest River	False Cape
1. Hopewell	.99826	.97109	.99932	.97664	.98925
2. Newport News		.96390	.99880	.96840	.98229
3. Sea Shore			.97118	.99918	.99434
4. Gum Swamp				.97601	.98847
5. Northwest R.					.99731

DISCUSSION

The average heterozygosity value of 1.6 reported here for Virginia populations of *A. piscivorus* is less than that of 4.1% - 8.3% reported for *Thamnophis sirtalis* by Sattler and Guttman (1976), and the 9.2% and 7.7% reported for *Thamnophis proximus* and *Thamnophis sauritus*, respectively, by Gartside et al. (1977). It is interesting to note the extremely low heterozygosity in the Sea Shore State Park population (0.9%). Virginia populations of *A. piscivorus* appear to exhibit less variation than other species of snakes. Two specimens of the Florida Cottonmouth, *Agkistrodon piscivorus conanti*, were found to be almost identical to Virginia specimens based on electrophoretic analysis of the same loci. Only the presence of a unique allele at the Got-1 locus distinguished it from Virginia *A. piscivorus*. Since there is so little variation between these two subspecies, the low amount of variation and heterozygosity reported in this study may be typical for the species. Thus, *A. piscivorus* may have had a rather conservative biochemical evolution, if we can draw inferences from data at hand.

The highest level of heterozygosity was observed in the population at False Cape State Park (2.9%), while the lowest was seen in the Sea Shore State Park sample (0.9%). This population is unique in that housing developments separate it from other populations. The surrounding area is one of the most rapidly developing areas in the United States. Although the park once supported high-density populations of cottonmouths, the species has become extremely rare there in recent years. Despite extensive field efforts over a two-year period, only two specimens were collected. This apparent decline may be an effect of the extensive droughts this area has suffered during the past few years.

Nei's (1972) index of genetic similarity reveals all populations are extremely close genetically. Even the lowest pairing value (.964), obtained in comparing Sea Shore State Park with the Newport News population, indicates a very high degree of genetic similarity among all Virginia populations sampled. Although all Virginia populations of *A. piscivorus* sampled are very similar genetically, the Hopewell, Gum Swamp, and Newport News populations show the highest values for genetic similarity. All three displayed a very high frequency for Xdh-1^a, which occurred in much lower frequencies in the other populations and was absent from the Sea Shore State Park samples. While the Newport News population shared a slightly higher identity with the Gum Swamp population than with the Hopewell population (.99880 vs .99826), this extremely small difference can be explained by the very low frequency of Got-2^a (.111) and higher frequency of Xdh-1^a (.944) in the Hopewell population. Both of these loci appear to be heading towards complete fixation of alleles, either by selection or by drift, in this isolated population. Such patterns

have been observed in isolated populations of other species (Avise 1976). Fixation of Xdh-1^b in the Sea Shore State Park population also appears to be in progress. Additionally, the presence of Lap-1^a in only the Hopewell and Newport News populations indicates the close genetic relationship between them. This suggests that the populations of *A. piscivorus* north of the James River are derived from populations in the vicinity of Hopewell rather than farther down the river. This hypothesis is supported by other species of snakes with distributions similar to that of the cottonmouth. *Nerodia taxispilota* occurs on the Peninsula, but does not extend to its lower end. The single Virginia record for *Regina r. rigida* is also from the uppermost reaches of the Peninsula. Whether this represents a relict population or a short-lived introduction is problematical. While these species would be able to cross the James River at its widest expanse, they are absent along the river in its lower reaches.

The distribution of the species least likely to cross large expanses of water, *C. horridus*, lends further credence to the hypothesis. The timber rattlesnake has a more extensive distribution on the Peninsula than does the cottonmouth, but it also is not recorded from the counties directly below the James River. Yet there are records for this species from Prince George County, very near the Hopewell population of *A. piscivorus*. Both *N. taxispilota* and *N. e. erythrogaster* also persist in this same general region.

The elimination of intervening populations for these species may be a result of climatic factors, at least in the case of *A. piscivorus*. It appears that the distributional range of this species in Virginia is contracting. Richard Hoffman (in Russ 1973) reported that cottonmouths were once common east of the Fall Line in Virginia, but are now only rarely found in many areas. Today, even in the Dismal Swamp, the species is so rare that the Park Service will not issue permits to collect this species. Many areas that once supported large populations no longer do so, and it appears that the range of this species is being pushed to the southeast. Blem (1981) felt that the record cold winters during the last decade may have had a decimating effect on the survival of this species at the northern edge of its range.

In summary, it appears that the levels of genetic variation observed in six Virginia populations of *A. piscivorus* are lower than those reported for other snakes. This species and several others of the Coastal Plain probably had more extensive distributions in the past, but appear now to be undergoing range contractions. Virginia cottonmouth populations that still seem to be thriving also display the highest genetic variation.

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LITERATURE CITED

Avise, John C. 1976. Genetic differentiation during speciation. Pp. 106-122 *in* Molecular Evolution. F.J. Ayala (Ed.). Sinauer Assoc., Sunderland, MA.

Blem, Charles R. 1981. Reproduction of the Eastern Cottonmouth *Agkistrodon piscivorus piscivorus* (Serpentes: Viperidae) at the northern edge of its range. Brimleyana 5:117-128.

Conant, Roger. 1975. A Field Guide to Reptiles and Amphibians of Eastern and Central North America. 2nd ed. Houghton Mifflin Co., Boston. 329 pp.

Engeling, Glen A. 1969. Reptiles and amphibians of York Co., and the Newport-Hampton area. Va. Herpetol. Soc. Bull. *62*:1-3.

Gartside, Donald F., J. S. Rogers and H. C. Dessauer. 1977. Speciation with little genic and morphological differentiation in the ribbon snakes *Thamnophis proximus* and *T. sauritus* (Colubridae). Copeia 1977(4):697-705.

Linzey, Donald W., and M. J. Clifford. 1981. Snakes of Virginia. Univ. Virginia Press, Charlottesville. 159 pp.

Nei, Masatoshi. 1972. Genetic distance between populations. Am. Nat. *106*:283-292.

Russ, William P. 1973. The rare and endangered terrestrial vertebrates of Virginia. Unpubl. M.S. thesis, Va. Polytech. Inst. State Univ. 339 pp.

Sattler, Paul W., and S. I. Guttman. 1976. An electrophoretic analysis of *Thamnophis sirtalis* from western Ohio. Copeia 1976(2):352-355.

Selander, Robert K., and W. E. Johnson, 1973. Genetic variation in vertebrate species. Annu. Rev. Ecol. Syst. *4*:75-91.

Selander, Robert K., M. H. Smith, S. Y. Yang, W. E. Johnson and J. B. Gentry. 1971. Biochemical polymorphisms and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse *Peromyscus polionotus*. Stud. Genet. VI. Texas Univ. Publ. 7103:49-90.

Wood, John T. 1954. The distribution of the poisonous snakes in Virginia. Va. J. Sci. *5*:152-167.

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